PATENT
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APPLICANT(S):	Kalle SAKSELA et al.		
APPLICATION NO.:	09/579,894	GROUP:	1627
FILED:	May 26, 2000	EXAMINER:	B. Celsa
FOR:	METHODS AND MATERIALS FOR GENERATING SH3 DOMAINS WITH TAILORED BINDING PROPERTIES		

DECLARATION SUBMITTED UNDER 37 C.F.R.§1.132

Honorable Commissioner of Patents
Washington, D.C. 20231

I, Dr. Marius SUDOL do hereby declare the following.

I am an associate professor in Mt. Sinai School of Medicine, New York, NY. I am a researcher in the field of protein domains and I am an inventor on two USA patents. I obtained my Ph.D. degree at the Rockefeller University in New York City, USA. Dr. Kalle Saksela was a colleague of mine from the tenure at the Rockefeller University.

In the recent past I had a chance to analyze some of the patent proceedings of Dr. Saksela. Based on my analysis I would like to state the following: The capacity of the RRT-SH3 domains revealed in the publication from the Saksela lab (Hiipakka et al., 1999 J. Mol. Biol. 293:1097-1106), the subject matter of which corresponds to the above-identified patent application, is novel and cannot be directly compared to the finding published earlier by the laboratory (Lee et al., 1995, EMBO J. 14:5006-5015). In my judgement the idea of generating artificial, ligand-tailored SH3 domains became obvious only from the publica-

tion of Hiipakka et al., 1999 and not from the previous report of Lee et al. from 1995.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Marius Sudol

Dr. Marius SUDOL

March 1, 2004

Date

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Exhibit A

An alignment showing approximately half of all SH3 domains found in the human genome. The positions of the five beta-strands of the SH3 protein fold are indicated with "b", and are separated by slashes ("-") corresponding to the loop regions or by a 3_{10}-helix ("3"). The first loop region is known as the RT-loop. The RT-loop consist of a region that is variable in sequence and length (typically six amino acids but ranging among the SH3 domains shown below from 3 to 14) flanked by four and eight relatively well-conserved residues. The variable region of the RT-loop (colored yellow) can be easily identified in the sequence of different SH3 domains based on the adjacent conserved amino acid motifs "ALYDY" and "DL" (colored green). The "ALYDY motif" almost invariably consists of a combination of the amino acids A/V - L - Y/F/W/H - D - Y/F/H, whereas the "DL motif", almost invariably consist of a combination of the amino acids D/E-L/I/V.

The present invention is based on our observation that by replacing the variable region of the Hck-SH3 domain of (EAIHHE; residues 69-74 of Hck protein) with completely random six-amino substitutions artificial SH3 domains (RRT-SH3 domains) can be generated that can bind with unnaturally high affinities to various ligands of natural SH3 domains despite the fact that their engineered RT-loop sequences bear no resemblance to natural SH3 domains. It is important to note, that we have successfully used Hck-derived RRT-SH3 domains to target with high affinity also ligands that (unlike HIV Nef) normally show no binding to Hck-SH3.

Although our Hck-derived RRT-SH3 library (carrying approximately 64 million different variants) appears sufficient for targeting almost any SH3 ligand protein (and thus to develop competitive inhibitors for virtually any SH3 domain), most if not all of the other SH3 domains could also be used as scaffolds for generating similar RRT-SH3 libraries. This could be done by replacing the variable part of the RT-loop (as defined above and colored yellow in the alignment below) by a stretch of random residues. Although this was the case in our Hck-derived RRT-SH3 library, the length of the randomized sequence would not need to be the same as in the original SH3 domain, and could range anywhere between 4 and 20.

```
    ALYDY              DL
    V F F              EI
      W H               V
      H
bbbbb-------------------------bbbbbb-------------bbbbbb------------bbbb3333bbbb
IIVVALYDYEA--------IHHEDLSFQKGDQMVVLEES----------GEWWKARSL--------ATRKEGYIPSNYVARV      Hck
TLFVALYDYEA--------RTEDDLSFHKGEKFQILNSS---------EGDWWEARSL--------TTGETGYIPSNYVAPV      Fyn
ELVLALYDYQE--------KSPREVTMKKGDILTLLNST-NKARHREWLCDWWKVEVN----------DRQGFVPAAYVKKL      alpha-II spectrin
QRVMALYDFQA--------RSPREVTMKKGDVLTLLSSI---------NKDWWKVEAA----------DHQGIVPAVYVRRL      alpha-I spectrin
NLVIALHSYEP--------SHDGDLGFEKGEQLRILEQS----------GEWWKAQSL--------TTGQEGFIPFNFVAKA      Lck
DIVVALYPYDG--------IHPDDLSFKKGEKMKVLEEH----------GEWWKAKSL--------LTKKEGFIPSNYVAKL      Lyn
HVVQALYPFSS--------SNDEELNFEKGDVMDVIEKP-------ENDPEWWKCRKI---------NGMVGLVPKNYVTVM      Nck1 #3
PQCKALYDFEV----KDKEADKDCLPFAKDDVLTVIRRV---------DENWAEGMLA----------DKIGIFPISYVEFN      POSH#2
EWCEALHSFTA--------ETSDDLSFKRGDRIQILERL---------DSDWCRGRLQ----------DREGIFPAVFVRPC      SH3d19 #4
MEAVAKFDFTA--------SGEDELSFHTGDVLKILSNQ----------EEWFKAELG----------SQEGYVPKNFIDIQ      GADS/Grp2 #1
PHAVVLHDFPA--------EQVDDLNLTSGEIVYLLEKI---------DTDWYRGNCR----------NQIGIFPANYVKVI      SH3d19 #2
RIFVALFDYDPVSMSPNPDAGEEELPFREGQILKVFGDK--------DADGFYQGEGG----------GRTGYIPCNMVAEV      PRAX-1 #2
NTYVALYKFVP--------QENEDLEMRPGDIITLLEDS---------NEDWWKGKIQ----------DRIGFFPANFVQRL      Stac
TIFVALYDYEA--------RTTEDLSFKKGERFQIINNT---------EGDWWEARSI--------ATGKNGYIPSNYVAPA      Yes
SQVEALESYEA--------TQPEDLEFQEGDIILVLSKV---------NEEWLEGECK----------GKVGIFPKVFVEDC      p67-phox #2
IEVIALYSFEG--------QQPGDLNFQAGDRITVISKT-------DSHFDWWEGKLR----------GQTGIFPANYVTMN      Homology to Grb2
HFVVALYDYTA--------MNDRDLQMLKGEKLQVLKGT----------GDWWLARSL--------VTGREGYVPSNFVARV      Blk
TTFVALYDYES--------RTETDLSFKKGERLQIVNNT---RKVDVREGDWWLAHSL--------STGQTGYIPSNYVAPS      N-Src
TTFVALYDYES--------RTETDLSFKKGERLQIVNNT---------EGDWWLAHSL--------STGQTGYIPSNYVAPS      Src
HSMVAAFDYNP-QESSPNMDVEAELPFRAGDVITVFGGM--------DDDGFYYGELN----------GQRGLVPSNFLEGP      PRAX-1 #3
VCAEALWDHVT--------MDDQELGFKAGDVIEVMDAT---------NREWWWGRVA----------DGEGWFPASFVRLR      Asef / ARHGEF4
IQVKALYDFLP--------REPCNLALRRAEEYLILEKY---------NPHWWKARDR----------LGNEGLIPSNYVTEN      Txk
VCAEALWDHVT--------MDDQELGFKAGDVIQVLEAS---------NKDWWWGRSE----------DKEAWFPASFVRLR      Homology to Asef
VSAEAVWDHVT--------MANRELAFKAGDVIKVLDAS---------NKDWWWGQID----------DEEGWFPASFVRLW      ARHGEF9
GVIYALWDYEP--------QNDDELPMKEGDCMTIIHRE------DEDEIEWWWARLN----------DKEGYVPRNLLGLY      53BP2
GVAYALWDYEA--------QNSDELSFHEGDALTILRRK------DESETEWWWARLG----------DREGYVPKNLLGLY      ASPP1
GAVYALWDYSA--------EFGDELSFREGESVTVLRRD------GPEETDWWWAALH----------GQEGYVPRNYFGLF      RAI
PTVVALYDYTA--------NRSDELTIHRGDIIRVFFKD---------NEDWWYGSIG----------KGQEGYFPANHVASE      Homology to Tipd
RKARVLYDYDA--------ANSTELSLLADEVITVFSVV-------GMDSDWLMGERG----------NQKGKVPITYLELL      endophilin B1
RKARVLYDYEA--------ADSSELALLADELITVYSLP-------GMDPDWLIGERG----------NKKGKVPVTYLELL      endophilin B2
RKAKALYDFRG--------ENEDELSFKAGDIITELESV---------DDDWMSGELM----------GKSGIFPKNYIQFL      SH3d19 #5
EEYIALYPYSS--------VEPGDLTFTEGEEILVTQKD---------GEWWTGSIG----------DRSGIFPSNYVKPK      Intersectin 2 #3
GIAIARYDFCA--------RDMRELSLLKGDVVKIYTKM--------SANGWWRGEVN----------GRVGWFPSTYVEED      Vav3 #2
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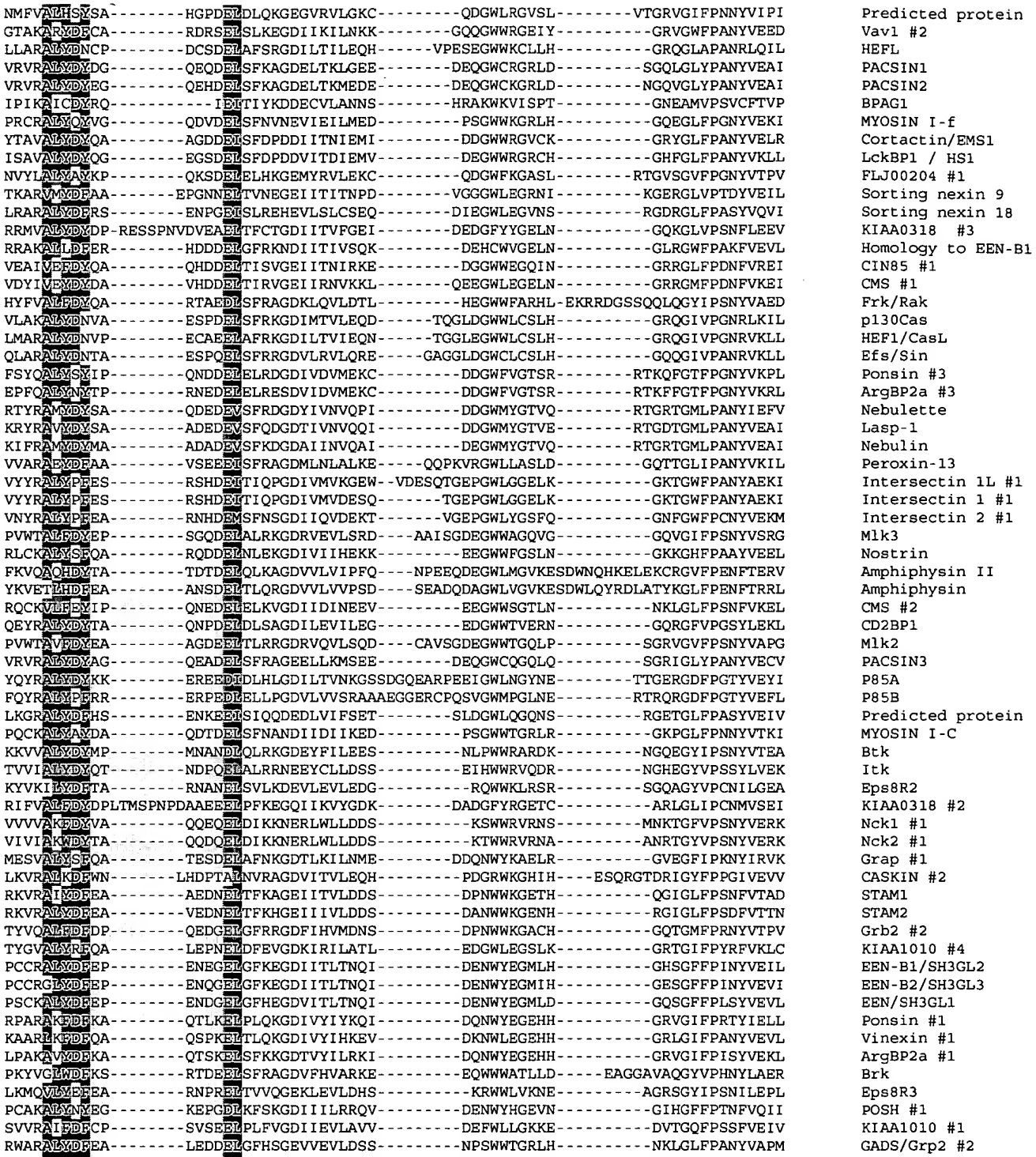
Predicted protein
Vav1 #2
HEFL
PACSIN1
PACSIN2
BPAG1
MYOSIN I-f
Cortactin/EMS1
LckBP1 / HS1
FLJ00204 #1
Sorting nexin 9
Sorting nexin 18
KIAA0318 #3
Homology to EEN-B1
CIN85 #1
CMS #1
Frk/Rak
p130Cas
HEF1/CasL
Efs/Sin
Ponsin #3
ArgBP2a #3
Nebulette
Lasp-1
Nebulin
Peroxin-13
Intersectin 1L #1
Intersectin 1 #1
Intersectin 2 #1
Mlk3
Nostrin
Amphiphysin II
Amphiphysin
CMS #2
CD2BP1
Mlk2
PACSIN3
P85A
P85B
Predicted protein
MYOSIN I-C
Btk
Itk
Eps8R2
KIAA0318 #2
Nck1 #1
Nck2 #1
Grap #1
CASKIN #2
STAM1
STAM2
Grb2 #2
KIAA1010 #4
EEN-B1/SH3GL2
EEN-B2/SH3GL3
EEN/SH3GL1
Ponsin #1
Vinexin #1
ArgBP2a #1
Brk
Eps8R3
POSH #1
KIAA1010 #1
GADS/Grp2 #2
Crk #1
CrkL #1
SRGAP2
SRGAP1
SRGAP3
Intersectin 2 #5
Intersectin 2 #5
PLC-gamma-2
PLC-gamma-1
HIP-55
AblBP3
Abi-1
Mlk4

EAHRVLFGFVP--------ETKEELQVMPGNIVFVLKKG---------NDNWATVMFN----------GQKGLVPCNYLEPV p67-phox #1